Claim Listing:

This listing of claims will replace all prior versions and listings of claims in the application.

1. (Currently Amended) A method for promoting auto-induction of transcription of cloned DNA in ~~cultures of~~ bacterial cells grown batchwise, which cells are adapted for use in expression of cloned DNA, said transcription being under the control of a promoter whose activity can be induced by an exogenous inducer whose ability to induce said promoter is dependent on the metabolic state of said bacterial cells, the method comprising:

a) providing a culture medium comprising:

i) an inducer that causes induction of transcription from said promoter in said bacterial cells;

ii) a metabolite that prevents induction by said inducer, said metabolite adjusted to a ~~the~~ concentration ~~of said metabolite being adjusted so as~~ sufficient to substantially preclude induction by said inducer ~~in the early stages of~~ until such time as growth of the bacterial ~~culture~~ cells depletes ~~but such that~~ said concentration of said metabolite ~~is depleted~~ to a level that ~~allows~~ permits induction by said inducer ~~at a later stage of growth,~~; ~~and~~

b) inoculating the culture medium with a bacterial inoculum, the inoculum comprising the bacterial

cells containing cloned DNA, the transcription of which is ~~induced~~ inducible by said inducer; ~~and~~

c) incubating the culture of step b) under conditions appropriate for growth of the bacterial cells; and

d) continuing to incubate the culture of step c) until depletion of the metabolite has permitted auto-induction of transcription.

2. (Currently Amended) The method of Claim ~~1~~ 45 wherein the bacterial cells are Escherichia coli cells.

3. (Currently Amended) The method of Claim 2 wherein the Escherichia coli cells are selected from the group consisting of BL21(DE3), B834(DE3) and HMS174(DE3).

4. (Original) The method of Claim 1 wherein the cloned DNA is carried in a plasmid expression vector.

5. (Original) The method of Claim 4 wherein the plasmid expression vector carries a T7lac promoter.

6. (Original) The method of Claim 1 wherein the promoter whose activity is induced by an exogenous inducer is repressed by the *lac* repressor.

7. (Currently Amended) The method of Claim ~~6~~1 wherein the promoter ~~whose activity is induced by an exogenous inducer~~ is selected from the group consisting of a *lac*

promoter, a *lac*UV5 promoter, ~~and~~ a T7*lac* promoter, and an araBAD promoter.

8. (Currently Amended) The method of Claim 1 wherein the exogenous inducer is selected from the group consisting of lactose, galactose, or arabinose.

9. (Original) The method of Claim 1 wherein the metabolite is glucose.

10. (currently amended) The method of Claim 1 wherein the metabolite is selected from the group consisting of glucose and amino acids or a combination thereof.

11. (Original) The method of Claim 1 wherein the culture medium further comprises a complex mixture of nutrients selected from the group consisting of yeast extract and a tryptic digest of casein.

12. (Original) The method of Claim 1 wherein the culture medium further comprises carbon sources that can be utilized by bacterial cells in the culture without preventing induction by the exogenous inducer.

13. (Original) The method of Claim 12 wherein the carbon sources are selected from the group consisting of glycerol, succinate, fumarate, malate, citrate, acetate, maltose and sorbitol.

14. (Original) The method of Claim 1 wherein the culture medium further comprises from about 0.5 mM to about 10 mM magnesium cation.

15. (Original) The method of Claim 1 wherein the culture medium further comprises from about 0.05x to about 2x metals mix.

16. (Original) The method of Claim 1 wherein the culture medium further comprises from about 5 mM to about 200 mM phosphate anion.

17. (Original) The method of Claim 1 wherein the culture medium further comprises from about 0.5 mM to about 25 mM sulfate anion.

18. (Original) The method of Claim 1 wherein the culture medium further comprises from about 20 mM to about 100 mM ammonium cation.

19. (Original) The method of Claim 1 wherein the culture medium further comprises from about 5 mM to about 200 mM sodium cation.

20. (Original) The method of Claim 1 wherein the culture medium further comprises from about 5 mM to about 200 mM potassium cation.

21. (Original) The method of Claim 1 wherein the culture medium comprises components such that the

culture after growth to saturation has a pH between about pH 4.5 and about pH 9.5.

22. (Original) The method of Claim 21 wherein the culture after growth to saturation has a pH preferably between about pH 5.5 and about pH 7.5.

23. - 39. (Cancelled)

40. (Currently Amended) The method of Claim 1 wherein the culture medium is selected from the group consisting of: ZYP-5052, PA-5052, P-5052, PASM-5052, MAS-15052, MS-15052, ~~and~~ ZYM-15052, and combinations thereof.

41. (New) The method of Claim 1 wherein the transcripts of the cloned DNA are translated.

42. (New) The method of Claim 1 wherein the culture medium further comprises a mixture of 18 to 20 of the natural amino acids.

43. (New) The method of Claim 42 wherein the mixture of amino acids comprises 18 of the 20 natural amino acids.

44. (New) The method of Claim 43 wherein the mixture of 18 amino acids excludes cysteine and tyrosine.

45. (New) The method according to Claim 1 wherein the bacterial cells are selected from the group consisting

of Escherichia coli, Bacillus subtilis, Ralstonia eutrophus, Salmonella enterica serovar Typhimurium, Pseudomonads and Rhodobacter capsulatus.

46. (New) A method for promoting the transcription of a cloned DNA by auto-induction of an inducible promoter in cultures of bacterial cells grown batchwise, which cells are adapted for use in expression of cloned DNA, the method comprising:

a) providing a culture medium comprising:

i) an inducer capable of inducing transcription from said inducible promoter;

ii) a metabolite that prevents induction by said inducer, said metabolite adjusted to a concentration which substantially precludes induction by said inducer until such time as growth of the bacterial cells depletes the concentration of said metabolite to a level which permits induction by said inducer,

b) inoculating the culture medium with a bacterial inoculum, the inoculum comprising the bacterial cells containing cloned DNA, the transcription of which is inducible by said inducer;

c) incubating the culture of step b) under conditions appropriate for growth of the bacterial cells; and

d) continuing to incubate the culture of step c) until depletion of the metabolite has permitted auto-induction of transcription by said inducer.

47. (New) The method according to Claim 46 wherein the bacterial cells are selected from the group consisting of Escherichia coli, Bacillus subtilis, Ralstonia eutrophus, Salmonella enterica serovar Typhimurium, Pseudomonads and Rhodobacter capsulatus.

48. (New) The method of Claim 47 wherein the cloned DNA is carried in a plasmid expression vector, which expression vector is adapted for use in said cells.

49. (New) The method of Claim 48 wherein the auto-induced transcripts are translated into protein.

50. (New) A method for promoting auto-induction of transcription of a cloned DNA in bacterial cells grown batchwise, which cells are adapted for use in expression of cloned DNA, said transcription being under control of an inducible promoter the induction of which can be blocked by at least one depletable culture medium constituent, the method comprising:

a) providing a culture medium comprising:

i) an inducer for said inducible promoter; and

ii) a concentration of at least one depletable culture medium constituent that can block induction by said inducer, the concentration being adjusted so as to substantially block induction by said inducer but such that growth of said bacterial cells depletes the concentration of the one or more constituents so as to relieve the block and

permit induction prior to growth of said cells reaching saturation;

b) inoculating the culture medium with an inoculum of said bacterial cells; and

c) incubating the inoculated culture of step b) under conditions appropriate for growth of said cells, depletion of said one or more constituents, and auto-induction of said transcription of said cloned DNA.

51. (New) The method according to Claim 50 wherein the cloned DNA is carried in a plasmid expression vector, which expression vector is adapted for use in said cells.

52. (New) The method according to Claim 51 wherein the auto-induced transcripts are translated into protein.

53. (New) A method for promoting auto-induction of transcription of cloned DNA in bacterial cells grown batchwise, which cells are adapted for use in expression of cloned DNA, said transcription being under the control of a *lac* promoter, the method comprising:

a) providing a culture medium selected from the group consisting of ZYP-5052, PA-5052, P-5052, PASM-5052, MAS-15052, MS-15052, ZYM-15052 and combinations thereof;

b) inoculating said culture medium with an inoculum of a said bacterial cells; and

c) incubating the inoculated culture medium of step b) under conditions for growth of said cells until a saturating cell density has been achieved.